

Human Neutrophil Migratory Function: Modulatory Effect of Interactions with Opsonized Particles

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Human polymorphonuclear neutrophils preexposed to cytotoxin or to phagocytizable particles exhibited reduced spontaneous and chemotactic migratory responses. This influence of cytotoxin appears to be related to toxic effects of by-products of hexose monophosphate shunt stimulation. To determine whether a phagocytic stimulus may inhibit subsequent neutrophil migratory functions by the same mechanism, we assessed spontaneous and chemotactic migratory functions of neutrophils from individuals with chronic granulomatous disease exposed to antibody-opsonized sheep erythrocytes. Our results showed that phagocytosis of such particles did not alter these migratory responses of chronic granulomatous disease neutrophils and suggest that phagocytic stimulation of normal neutrophils may modulate migratory function by some mechanism dependent upon hexose monophosphate shunt stimulation.

Neutrophil chemotaxis is a result of a series of membrane and cytoplasmic events presumably initiated by interaction of a chemotactic agent with its membrane receptor (14). It is possible to reduce a chemotactic response and spontaneous migration of human neutrophils by a variety of manipulations including preincubation with various chemoattractants (14, 16), phagocytizable particles (8), or immune complexes (7, 8). This consequence of preexposure of neutrophils to chemotactic agents has been termed "deactivation" by Ward and Becker (15).

The mechanism by which complement-mediated deactivation of rabbit peritoneal exudate neutrophils is thought to occur has been postulated to involve the activation and decay of a labile "activatable" esterase (15). Cytotoxin-mediated deactivation of human neutrophils has been attributed to the effects of secreted lysosomal enzymes (4) or of toxic by-products of hexose monophosphate shunt activity (1, 2). The observed failure of neutrophils from patients with chronic granulomatous disease (CGD) to undergo a cytotoxin-induced respiratory burst and to lose migratory function (3, 9) is evidence for the possible contribution of autooxidative reactions to the deactivation phenomenon.

Phagocytosis-mediated loss of human neutrophil chemotactic function has been tentatively

attributed to the concomitant loss of receptors for opsonin and chemotactic agents (7). However, since phagocytosis, similar to ligand binding, stimulates the respiratory burst (5, 6), we have reexplored the mechanism by which interactions with ingestible particles may modify the migratory functions of the neutrophil. To determine whether phagocytosis and binding of cytotoxin may share a common mechanism based upon stimulation of the respiratory burst, in effecting a loss of neutrophil migratory function, we compared the effect of exposure to opsonized erythrocytes on subsequent migratory responses of neutrophils from healthy donors and individuals with CGD.

MATERIALS AND METHODS

Patient population and preparation of cells. Five patients with CGD and five healthy controls were the blood donors. Neutrophilic granulocytes from the individuals with CGD who participated in this study have been studied previously in our laboratory and have been found to be abnormally responsive to manipulations which are known to stimulate oxidative metabolism. The venous samples were drawn in heparinized syringes, and the polymorphonuclear neutrophils (PMN) were isolated as buffy-coat leukocytes as previously described (11). These buffy-coat leukocytes were washed with minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) and suspended in the medium to provide a final concentration of 2.5×10^7 PMN/ml before transfer to the agarose plates.

Cell migration assay system. The chemotactic and spontaneous migratory functions of the PMN

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populations were assessed by the chemotaxis-under-agarose system as previously described (11). The chemoattractant used was *N*-formyl-methionyl-leucyl-phenylalanine (Andrulis Research Corp., Bethesda, Md.) at a concentration of 10^{-7} M. All plates were incubated for 3 h at 37°C in a 5% CO₂ atmosphere before fixation, staining, and assessment of cell migration.

PMN-erythrocyte mixtures. Two percent suspensions of nonopsonized erythrocytes (E) and immunoglobulin G-opsonized erythrocytes (EA) at 1:100 were prepared as described previously (7). PMN (5 μ l) were first placed in the center wells and then 5 μ l of E or EA (1:100) was immediately added to the center well PMN. This resulted in a ratio of five erythrocytes to one PMN. Control PMN were studied in parallel with CGD PMN for each experimental protocol.

All tests for both spontaneous migration and chemotaxis were conducted in triplicate, and the results are expressed as the mean projected migration distance in centimeters \pm standard error of the mean.

RESULTS

The spontaneous and chemotactic migratory functions of the CGD neutrophils studied did not differ significantly from these functions of leukocytes from healthy donors (Fig. 1). The data presented in Fig. 1 also show the effect of addition of E and EA on the migratory response of control and CGD neutrophils to *N*-formyl-methionyl-leucyl-phenylalanine. Addition of E neither enhanced nor inhibited the migratory response of neutrophils from either source. Addition of EA, however, reduced the chemotactic and spontaneous responses of control neutrophils by 44 and 25%, respectively, but did not affect either response of the CGD neutrophils.

To confirm that this lack of CGD PMN response to EA was consistent, two patients with CGD were restudied at a subsequent time. Cell migration values for CGD PMN were at similar

levels with and without exposure to EA (unpublished data).

To determine whether the interaction of CGD neutrophils and EA was markedly dissimilar from the control PMN, both CGD and control neutrophils were added to EA and incubated in sterile test tubes for 3 h at 37°C in 5% CO₂. These cell mixtures were then examined by light microscopy for the extent of rosette formation or phagocytosis. Phagocytosis was defined as at least one erythrocyte ingested and rosette formation as a minimum of three attached erythrocytes. The control ($n = 2$) and CGD ($n = 2$) PMN had mean phagocytic percentages of 32 and 35, respectively, whereas rosette formation was 55 and 59%, respectively.

DISCUSSION

We demonstrate in this report that neutrophil migratory functions were not altered directly by phagocytosis or adherence of opsonized particles. This is shown by the failure of CGD neutrophil interaction with EA to interfere with subsequent spontaneous or chemotactic migratory responses. The inability of this treatment to alter CGD neutrophil migration is not attributable to failure of these neutrophils from children with CGD to phagocytize or bind the EA.

We conclude from this observation that some consequence of phagocytosis or immune adherence must provide the mechanism by which these events interfere with migratory functions of neutrophils from healthy individuals. Since a respiratory burst is known to occur after phagocytosis or antibody receptor occupancy on the normal neutrophil (5, 6), and since normal neutrophils may lose migratory function nonspecifically, in part as a consequence of cytotaxin-induced stimulation of hexose monophosphate

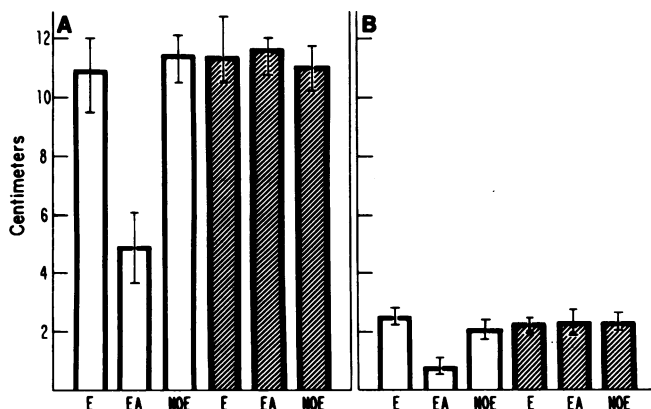


FIG. 1. (A) Chemotaxis and (B) spontaneous migration of normal neutrophils (□) and neutrophils from patients with CGD (▨) incubated without erythrocytes (NoE), E, and EA.

shunt activity (9), we thought it possible that phagocytosis- and cytotoxin-induced loss of migratory functions of normal neutrophils might share a common basis related to shunt activation. The observed lack of effect of EA on CGD neutrophil migratory functions supports this relationship since such neutrophils, lacking a critical oxidase activity, cannot be stimulated by usual means to undergo a respiratory burst (3). Alteration or disappearance of membrane receptors for chemotactic agents may not, as originally postulated (7), play a prominent role in phagocytosis-mediated loss of migratory function. Preliminary studies which demonstrate normal binding of *N*-formyl-methionyl-leucyl-[³H]phenylalanine to normal neutrophils after exposure to EA (Nelson et al., unpublished data) further support discounting the latter alternatives.

The effect of EA on both the spontaneous and chemotactic migratory responses of normal neutrophils and its association with stimulation of hexose monophosphate shunt activity make it appear that this phenomenon is analogous to the high-dose cytotoxin-induced nonspecific component of chemotactic deactivation we have recently described (10). In this case, loss of both migratory functions induced by phagocytosis and immune adherence would have a common basis, and loss of chemotactic function would then be attributable simply to loss of spontaneous mobility.

Although it is possible that other events which follow neutrophil-particle interaction, such as changes in chemotactic receptor affinity, cytoskeletal integrity (13), adherence properties (12), membrane esterase activity (15), or lysosomal enzyme secretion (4), may modulate cell movement, the interrelationship of oxidative metabolism and neutrophil migration appears significant. The mechanism of this relationship, its contribution to modulation and control of normal neutrophil migratory behavior, and the contribution of loss of this control mechanism to the pathology of chronic granulomatous disease deserve further investigation.

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